REMARKS

Status of the claims

Claims 28, 30-35 and 59-61 are pending in the application. Claims 28, 30-35 and 59-61 are rejected. Claim 62 is withdrawn. Claim 28 has been amended herein. No new matter is added.

Claim Amendments

Claim 28 has been amended to specifically recite "said two part probe structure thereby creates a linear overlap between the probe and the reporter linkers" to overcome 35 USC §103 rejection. The instant specification demonstrates that the linear overlap between the linker region of the probe overlap linker of the first oligonucleotide and the overlap linker of the second oligonucleotide of the two-component probe unit, stabilizes the probe and supports multiple or highly extended reporters. This unique structural design magnifies the binding forces between the target and the complex of reporter-probe-reporter because in this linear structural situation the binding between the base pairs of the linker sequences are additive. This structural configuration of the instant invention was intentionally and specifically designed to overcome the limitation of the probe structure of Wang et al and Urdea et al.

Claim rejection under 35 USC §103

Claims 28, 30-35 and 59-60 are rejected under 35 USC §103 (a) as being unpatentable over **Wang** et al (U.S. Patent 4,925,785 in view of **Urdea** et al (U.S. Patent 5,681,697). The Examiner states that **Wang** teaches a method for detecting a target nucleotide sequence similar to the instant invention. The Examiner states that **Wang** et al. teach all aspects of independent claim 28 except for use of Nucleic acid reporter arrays. The Examiner also states that **Urdea** et al. overcome this deficiency by teaching the use of reporter arrays to amplify signals for target detection. Further, Examiner states that **Urdea** et al. teach a first terminal probe linker and a second terminal probe linker (see figure 16, where LE has an X and Y region that hybridizes to the Amp 1 probe). Applicant respectfully disagrees. Applicant respectfully traverses this rejection.

Applicants submit that the methods of the instant invention comprise: 1) the hybridization of a two-component probe unit to the target and 2) the secondary hybridization of the reporters via the reporter linkers to the probe linkers of the probe; to create a linear complex of reporter-probe-reporter that is centrally wrapped around the target sequence. The reporters

linked via the linkers to the <u>two-component</u> probe unit are used for detection to indicate the target sequence and can be fluorescent, bioluminescent or any other molecule that can be used for detection. Hence, the instant invention teaches a nucleic acid probe unit consisting of <u>two overlapping oligonucleotide subunits joined together</u> to form a linear probe subunit. The first oligonucleotide has three segments sequentially: a first probe linker on one end (5') that hybridizes to the reporter linker of a reporter (but does not bind the single stranded target nucleotide sequence), a central sequence complementary to the target nucleotide sequence, and an overlap linker on the other end (3'). The second oligonucleotide of the probe unit consists of two segments sequentially: a matching overlap linker that is hybridized to the corresponding overlap linker on the first oligonucleotide, and a second probe linker, which hybridizes to a reporter linker of a reporter and is not complementary to the target sequence.

In contrast, the probe unit taught by **Wang** et al consists of a <u>single oligonucleotide, complementary to the target sequence (P_1, P_2....P_n, see Fig. 4A and example 2)</u> to which are attached universal sequences (A, B, C, D) that are complementary to universal sequences that are attached to a large random-coil polymer (a double stranded DNA), which acts as a reporter. Hence, **Wang's** probe unit consists of a central region (for example P_1 in Fig. 4A) complementary to the target sequence and two universal linker sequences on either ends of this central region (for example A and B in Fig. 4A).

There is no teaching or suggestion in **Wang** et al of a two-component probe unit. The Examiners quotes Fig. 4C and states that this is the nucleic acid probe unit where two oligonucleotides are overlapped. In actuality, Fig. 4C represents two independent probes (P_j) hybridized to a target sequence "T", via the central region complementary to the target sequence. The second probe (P_j) is attached to the reporter (a random-coil polymer) via the complementary universal linkers present on the probe (A and B) and those present on the reporter (A' and B'). Thus Fig. 4C does not represent the probe unit alone. The overlapped oligonucleotides, in Fig. 4C of **Wang** et al, are the linker sequences (A and B) present on the probe (P_j) and the linker sequences (A' and B') present on the reporter polymer, such that the overlap is between A and A', B and B'. The <u>A' region is the linker sequence of the reporter</u> and is not a part of the probe as is misinterpreted by the Examiner (the Examiner wrongly refers to A' as probe region). In addition, the Examiner refers to B' region as the B' region (linker) of the second oligonucleotide, which in **Wang's** case is the reporter (random-coil polymer) and is not a part of the probe unit.

The second oligonucleotide taught by the instant invention is part of the two-component probe unit and is not a reporter. Hence, structurally, the probe unit of the instant invention is entirely different than the probe unit taught by **Wang** et al.

Further, the probe unit of the instant invention is synthesized as two overlapping oligonucleotide subunits, which are joined together (Figure 3B). The first oligonucleotide of the probe unit has a small set of spacers incorporated (i.e. TTT) between the target specific sequence and the linker sequence to facilitate better hybridization. The second oligonucleotide, of the two-component probe unit, on its 3' end has the overlap linker sequence complementary to the second linker sequence of the first oligonucleotide. This region is designed with 5'TA sites to facilitate psoralen plus UV crosslinking to covalently link the two subunits together. The reporter associates with the two-component probe unit via its reporter linker sequence and the reporter linker sequence present on the first oligonucleotide of the two-component probe unit on one end and by associating via its reporter linker sequence with the reporter linker sequence present on the second oligonucleotide of the instant invention. In contrast, <u>**Wang** et al teach only a one-part single stranded probe</u>, generally comprising, <u>a target specific sequence</u> and a <u>linker sequence</u> for joining to a polymer based reporter unit, on one or both ends of the target sequence

Structurally, the **Wang** method teaches a collection of different target-specific probes in which each probe comprises one oligonucleotide or one single-stranded segment of DNA. Accordingly, the probes in the collection differ in their target-specific sequences, but they all share common linker sequences. Such single-stranded probes by definition comprise one 3' end and one 5' end. In contrast, the instant invention teaches nucleic acid probe unit specific to a single target sequence that consist of two oligonucleotides that are overlapped and joined via two matching end linkers. Thus, unlike **Wang** et al who teach multiple probes that comprise one single-stranded component with a 3' and a 5' end, the instant invention teaches a two-part probe.

The probes of the instant invention by definition comprise either two 3' ends or two 5' ends, and if the same linker sequence is employed at both ends, then the probe unit can bind two copies of the same reporter unit. In the examples reduced to practice and recited in the instant invention, the probe units are prepared with two identical 5' linkers and DNA-based reporters called GeneTAGs are prepared with matching 5' linker on one or both ends so that a batch of identical reporters can be employed to bind the probes, one copy of the reporter binding to each end of the probe after the probe is bound to the target. Alternatively, arrays of double linker reporters can be

employed with the probes of the instant invention. In contrast, the probe unit of **Wang** et al does not correspond to the structure and function of the probe unit of the instant invention.

The present invention is also characterized by another important structural and functional feature in that after hybridization of the probe to the target and following the hybridization of the dual reporters to the probe, the resulting complex of the dual terminal reporters and a central probe unit comprises a linear, <u>centrally wrapped reporter/probe/reporter complex that provides a partial helical enclosure of the target sequence</u> midway along the length of the reporter/probe/reporter complex. This structurally advantaged binding mechanism allows for tethering multiple long reporters on a single target site. In effect the instant invention has no practical limitations in terms of detecting a large or small number of target sequences since detection is based on detecting signal from the attached dual reporters or arrays of said reporters, which provide multiple fluorescent labels or other signals. The instant invention has the capacity to detect a single target site in a single cell and has been effectively reduced to practice (Example 12 of the instant invention), wherein a single probe is bound to a single target and multiple reporters are bound by linker ends of the probe to create multi-reporter, reporter-probe-reporter complex that provide a partial helical enclosure of the target sequence, whereas **Wang** et al. teach a series of different probes and thousands or millions of target to enable detection (Example 2 of **Wang** et al.). In addition, Applicants have amended claim 28 to recite "<u>said two part probe structure thereby creates a linear overlap between the probe and the reporter linkers.</u> It must be understood that the composition of the instant invention is specifically designed to create a highly advantaged capacity for binding reporters to the target by virtue of the two-component probe structure. For example, by creating a probe with two identical 5' linkers on each end, said two part probe unit will bind reporters with matching 5' linker ends on a end-to-end <u>linearly</u> overlapped basis wherein each reporter will similarly pull on the end linkers of the probe which is centrally wrapped around the target sequence <u>(page 27, 2nd paragraph, lines 9-23).</u> This structural design magnifies the binding forces between the target and the complex of reporter-probe-reporter because in this linear structural situation the binding between the base pairs of the linker sequences are additive. In contrast, the binding forces between target and probe-reporter in the case of a probe with one terminal linker are not additive. In the instant invention as practiced, the universal linkers are all 27 bases long, and thus the reporter binding capacity of such probes are approximately 27 times stronger than the reporter binding capacity of one linker probes such as the one linker probes typical of Urdea et al

and Wang et al. (All of this must be understood in the context of detecting targets *in situ* which are the focus of the instant invention, wherein probes and reporters are hybridized to an *in situ* bound target and then washed to remove unbound probes and reporters.) This major functional difference is true because, when a reporter is bound to a one linker probe, during washing the probe will be pulled laterally relative to the target such that each base pair is separated one by one. Thus washing must be very gentle to avoid releasing the probe from the target and thereby the reporters. In contrast, with the instant invention, once reporters are bound to each end of the probe after the probe has been wrapped around the target, the binding forces between probe and target becomes irrelevant, and the reporters are effectively tethered to the target. Now the forces of the wash step will only affect the binding forces between the overlapped linkers of the probe and reporters which are lined up end to end, thus summing the 27 base pair binding forces between each universal linker segment. This is why a series of reporters can be bound to a single target with this method. The reporters bound to each end of the probe counterbalance one another and do not pull the probe from the target. This structural configuration of the instant invention was intentionally and specifically designed to overcome the above described limitation of the probe structure of Urdea et al. This same structural limitation is also true of Wang et al. in the configurations reduced to practice. It is only in the case of Fig. 4C of Wang that there appears to be a probe which can bind reporters from both ends, except that Wang et al. describes a single oligonucleotide probe with both a 3' and a 5' end and thus said probe cannot bind two copies of same reporter on the same basis as the two part probes and reporters of the instant invention. Indeed, it is clear that Wang et al. did not intend and did not achieve any structural advantage in that aspect of that invention, because it was not designed or applied to the detection of in-situ targets which are in fact the targets of the instant invention and which are greatly advantaged in detection due to the structural features herein described. This method is comparable to the structural advantage of Velcro fasters, which are structurally advantaged when overlapped end-to-end and pulled linearly vs. laterally. The same principle of physics applies to the instant invention relative to Urdea et al. and Wang et al. Lateral pulling on overlapped Velcro fasteners will easily open them one hook and eye at a time, whereas linear end-to-end pulling strongly resists opening because the binding between hooks and eyes in that configuration are summed together. This is what similarly occurs during washing in the case of the instant invention where the wrapped reporter-probe-reporter complex shifts the binding issue to the linear overlap between probe and reporter linkers. This situation contrasts with the weak lateral

binding forces between probe and target that occurs with one-linker probes during wash steps. For example, Urdea et al. describe HIV detection with bDNA probes wherein about 27 different probes must be employed stepping along the genome, each binding a set of reporters in order to detect the target, whereas the instant invention is capable of detecting a similar target in situ with a single wrapped probe plus reporters. The instant invention is further advantaged by having identical 5' linkers at each end of the probe so that only one reporter species is needed since multiple copies of said reporter will bind equivalently to each end of the wrapped probe. Thus, the method of **Wang** et al differs considerably from the instant invention in both structural components and in the sequential steps of making and using the probes, the structural and functional characteristics of the probe and the dual reporter complex, and the potential applications of that reporter-probe-reporter structure.

Furthermore, the instant specification teaches reporters that are labeled double stranded DNA with single stranded linker regions on one or both ends that have the same polarity as the terminal linkers of the probes. Thus the same reporter unit can be attached to both ends of the two-part probe unit via complementary base pairing between their respective terminal linkers to form a linear reporter/probe/reporter unit that is centrally bound to the target sequence (Fig. 3A, 3B, and 3C). **Wang** teaches reporters that are random-coil polymers of high molecular weight such that each polymer molecule occupies large hydrodynamic volumes in solution (Column 7, lines 6-14). These polymers are selected from a group but not limited to commercially available synthetic polymers like polystyrene sulfonic acid, polystyrene malic anhydride, polysaccharides, polyglutamic acid, polyamino acid etc. (Column 7, lines 15-25). Biological polymers are also taught by **Wang** and include large DNA and RNA molecules. In addition, the detection of hybridization in **Wang** is based on whether the polymer is labeled or not. In the homogeneous assays, specifically Fig. 4C of **Wang** et al, as quoted by the Examiner, the polymer is not labeled but is attached to the probe, hybridization is detected based on changes in viscosity or gelation due to network formation. The method of **Wang** et al is distinguished from the instant invention in that the detection of hybridization in **Wang** et al has practical limitations since there is a requirement for a measurable high viscosity or gelation i.e., many probe –reporter and reporter-reporter cross-links have to form and each cross-linked species has to have a high molecular weight, hence this methodology is only suitable for the detection of a large number of target sequences, and generally multiple different probes are required, each binding a different site within the target region (Example 2). In contrast,

the double-stranded DNA- based reporters of the instant invention are detected via labeled or modified bases that are incorporated into the reporter unit.

Claims 30 and 31 of the instant invention teach a <u>labeled reporter</u> comprising of a double stranded polynucleotide sequence linked on one or both ends to a linker and is at least 100 bases long. **Wang** et al teach high molecular weight random coil polymers as the reporter, which depending on the assay is not necessarily labeled. Particularly, the Examiner refers to Fig. 4C, in which the reporter is an unlabeled large random-coil polymer and the detection is based on changes in gelation or viscosity due to formation of extensively cross-linked networks (Example 2). **Wang** et al also teach that the high hydrodynamic volume provided by the high molecular weight random-coil polymer is an advantage as it facilitates better detection both in homogenous as well as heterogenous assays.

With regard to claim 32 of the instant invention, **Wang** teaches formation of a reporter array with <u>multiple probes</u> whereas the instant invention teaches formation of a reporter array via complementary base pairing within the reporter linker regions between <u>multiple reporters</u>. The instant specification teaches a probe unit synthesized as two overlapping oligonucleotide subunits joined together. The primary probe unit contains a first linker sequence on the 5' end which is complementary to the reporter linker sequence, followed by a target specific sequence in the middle, and a second linker sequence in the 3' end which is complementary to a second probe subunit. The <u>second probe unit is generic and lacks the target specific sequences</u>, while its 3' end is complementary to the second linker sequences of the primary probe subunit, and its 5' end contains the first linker sequence complementary to the reporter unit. This probe is distinguished by the initial formation of a probe that wraps around the target sequence at mid-point and by secondary binding of the reporters to the linkers. Hence, the array built up is due to attachment of multiple reporters to one probe thus amplifying the signal from the initial probe and reporter hybridization to increase the sensitivity of detection.

To conclude, **Wang et al** teach a probe that is a single stranded piece of DNA or RNA, and not two overlapped oligonucleotides as taught by the instant invention. Further, the so called universal sequences or linkers taught by **Wang et al** are a low complexity, highly repeated sequence which is greatly different from the unique, high complexity linkers of the present invention that also include multiple 5'TA sites for psoralen/UV crosslinking. Furthermore, the linkers taught by **Wang et al** are joined to the probe molecules by end ligation, as contrasted

with the linkers of the instant invention, which are synthesized as a terminal part of the probe components. In addition, the reporter taught by **Wang** et al is a random coil polymer generally of polystyrene that can bind to itself as well as a probe, in order to create a large hydrodynamic volume in solution, a process that causes local gelatin, the means of detection, and DNA or RNA molecules are considered substitutes for such chemical polymers, and if so, such DNA –based reporters are depicted by a double line if double stranded, which is the case of the segments of Fig. 4C identified as B' to D' or as A' to C'. Thus those segments are double stranded reporter molecules, added to the single stranded probe after it has hybridized to the target and washed, and there can be no basis for describing the DNA segments B' to D' or A' to C' as a second oligonucleotide component overlapped with the first oligonucleotide.

Urdea et al teach methods to reduce background signal in nucleic acid hybridization assays. **Urdea** et al do not teach methods of detection of target nucleic acid but rather **Urdea** et al teach detection and reduction of signals that arise due to undesirable interactions that give rise to a signal which does not correspond to the presence of target nucleic acid. Hence, the methods taught by **Urdea** et al increase both the sensitivity and specificity of nucleic hybridization assays, <u>by reducing the incidence</u> of signal generation that occurs in the absence of target. On the contrary, the instant invention focuses on teaching methods for the detection of target nucleic acid. Furthermore, the probe (LE) taught by **Urdea** contains a L-1 region complementary to the target sequence and a L-2 region complementary to the label reporter or amplifier. This is consistent with what is shown in Figures 8, 11-13, and 15. Similarly, Figure 16 shows one LE probe with one terminal linker X and another LE probe with one terminal linker Y. The second vertical line connected to the LE probe in Figure 16 is not labeled as terminal linker and is not hybridized to any label reporter or amplifier. Hence, Figure 16 should not be interpreted as teaching two terminal linkers at both ends of the probe.

Since **Urdea** et al teach a significantly different probe composition than that of the instant invention hence, the rest of the teachings of **Urdea** et al. cannot apply to the instant invention. Hence, a combination of the teachings of **Urdea** with **Wang** et al cannot make the instant invention obvious since **Wang** et al, as discussed supra, also teach a significantly different probe composition. Hence, **Wang** and **Urdea** combined do not render the instant invention prima facie obvious as they do not teach or suggest all the claim limitations. Thus the combined teachings of

Wang and **Urdea** would not provide, one of ordinary skills in the art, a reasonable expectation of success of arriving at the instant invention.

Accordingly, in view of the amendments and the arguments presented herein, Applicants respectfully request that the rejection of claims 28, 30-35 and 59-60 under 35 U.S.C. 103(a) be withdrawn.

This is intended to be a complete response to the final Office Action, mailed December 12, 2006. Applicants submit that pending claims 28, 30-35 and 59-61 are in condition for allowance. If any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

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ADLER & ASSOCIATES
8011 Candle Lane
Houston, Texas 77071
Tel: (713) 270-5391
Fax: (713) 270-5361
badler1@houston.rr.com

Benjamin Aaron Adler, Ph.D., J.D.
Registration No. 35,423
Counsel for Applicant